

NUMBER OF LARVAL INSTARS OF *SIMULIUM* (*PSILOPELMIA*) *HAEMATOPOTUM* (DIPTERA: SIMULIIDAE)¹

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ABSTRACT: *Simulium (Psilopelmia) haematopotum* Malloch is a noxious haematophagus species distributed from Mexico to the West Indies to Guyana. Six larval instars were obtained by measuring the length of the postgena of 1203 specimens collected from the Balsas River of Mexico, from December 1983 to May 1984.

The knowledge of the life history and size relationships of black fly larvae and their susceptibility to different control methods (Habib 1983) are important data when a control program is planned (de Moor 1982). Also, it is necessary to establish the different larval instars when more than one generation coexists, or when different egg-laying times occur, or when there is the possibility of an overlapping of sibling species.

Larval instars of insects are usually separated on the basis of the size of some sclerotized structures (Dyar 1980). The most frequently measured structure is the head capsule using as indexes the total width, width of the cephalic apotome (frontoclypeus), and the lateral length of the head. Several studies on the number of larval instars in the Simuliidae have been carried out in the Neotropical Region (Ross and Merritt 1978; de Moor 1982; Coscarón and Bramardi 1996; Hamada 1989; Costa Jtabere da Cunha 1995). The number of instars of *Simulium haematopotum* ranges from 4 to 9, while most other species studied have 6 to 9 instars (Colbo and Okaeme 1988).

The objective of the present study was to determine the number of larval instars of *Simulium haematopotum* by various measurements of the larval head capsule. This is the only black fly species found in the Balsas River at the municipalities of Ciudad Altamirano, and Coyuca de Catalan, Guerrero State, Mexico. At these localities the black fly larvae survive very well in the slow flowing water. This species is considered part of the epilithon fauna although it is usually found on any smooth surface such as trailing grass, dead plants, glass bottles, and plastic items. Larvae can be found from the surface to 70 cm in depth. They are present throughout the year, but the population decreases from May to June.

The sampling area was the Balsas River basin, between the cities of Ciudad Altamirano and Coyuca de Catalan (18° 21' N and 100° 39' W), at an altitude

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up to 250m. The climate follows Koppen's climatic classification modified by Garcia (1981), and is warm subhumid with summer rainfalls and only one drought in winter, and has an annual average temperature of 28° C. May is the warmest month (32° C) and December the coldest (25° C). The annual average rainfall is about 1,010 mm and July is the rainiest month with 256 mm. There is no rainfall in February and March (Garcia 1981) The vegetation corresponds to deciduous tropical forest, but the irrigation of crops now prevails. The Balsas River is a unique permanent water source in the study area.

MATERIALS AND METHODS

Twenty monthly samples were taken from the Balsas River from December 1983 to May 1984. Black fly immatures were fixed in 80% ethanol for future identification and measurement. The number of larval instars was determined following the methodology proposed by Fredeen (1976, 1981) where the length of the postgena was the structure measured. All 1,203 specimens were measured using a stereomicroscope with an ocular reticule. All specimens are deposited in the Museo de La Plata, Universidad Nacional de La Plata (Argentina).

Data from these measurements were grouped following the procedures of Sokal and Rohlf (1960). According to this methodology in a polymodal frequency, the individual peaks identify data populations corresponding to different larval instars (number of larvae vs length of the postgena). In order to verify the precision of the groupings of larval instars, the Crosby growth rule (de Moor 1982), and the "t" test of Students for means comparison, were used (0.05 significant limit). The Crosby rule states that if there is a difference bigger than 10% between two consecutive Brook Ratios there is a possibility that an instar is missing.

The Brook Ratios represents the relationship $rB1 = Me2 / Me1$, where $Me1$ = means measurements of the parameter (postgena length) in the previous instar. $Me2$ = means measurements of the parameter in the following instar. rB = relationship between Me previous and later.

Crosby's Rule $(rB2 - rB1/rB1) 100 < 10\%$

The relationship between the larval instars and the length of the postgena was determined by means of lineal adjustments and potentials for each specimen through the procedure PROC REG of the SAS statistical program. The results showed that the potential regression was the unique factor that offered a better adjustment of data (a bigger coefficient value for the determination of R^2 , smaller residual error and aleatory behavior of the residual ones). On the residual of each adjustment Levene's test was applied for the verification of the homogeneity variances. As this supposition was not observed for the application of the regression technique, it was decided to carry out the



Fig. 1. Map showing the collection areas A: Balsas River; B: Cutzamala River; C: El Espiritu River; 1: Cutzamala town; 2: Ciudad Altamirano; 3: Coyuca de Catalan town; 4: Tlapehuala town.

adjustments considering the inverse of the variances.

RESULTS AND DISCUSSION

The number of larval instars was determined by the distribution of the frequencies of the length of the postgena. The distribution of the frequencies in the length of the postgena of the 1,203 larvae analyzed is shown in Fig. 2. The presence of 5 distinct larval instars can be seen ($p < 0.05$). The regression analysis (Fig. 3) indicates that the relationship between the number of larval instars and the lengths of the postgena corresponds to the equation:

$$y = 108.45 e^{0.2392x}; (r^2 = 0.82)$$

"Y" = postgenal length; "x" = instars

The presence of a first instar that doesn't appear in the distribution of frequencies should be kept in mind. This instar is recognized by the impression of the contact "raptor ovi" in the middle of the cephalic apotome and antennae without apparent divisions. It is an instar of short duration because the first instar passes to the second instar shortly after eclosion. It rarely appears in the samples from the river.

The application of the Crosby rule (Table 1) allows us to corroborate the number of instars as determined by the distribution of frequencies. The values of the ratios from the Brook comparison of instars 2 and 3 are slightly higher. This is due to the fact that instar 2 possesses a very small head capsule with hypostomal and postoccipital margins poorly defined. Using an increase of 67.2, an error of measurement of 0.5 units is possible, and this would cause an error of measurement of approximately 10. Owing to this fact, the anomalous values could be less than 10% taking into account a measuring error.

Through this, we consider *Simulium (Psilopelmia) haematopotum* to have 6 instars where the cephalic lengths correspond to the instars as follows: 2nd: 137-206; 3rd: 206-251; 4th: 251-320; 5th: 320-411; 6th: 411-457.

Table 1: Mean postgenal length, Brook's ratio and Crosby's rule for *S. (P.) haematopotum*.

Instar	Mean	Brook's ratio	Crosby's rule
II	171.43	1.399	11.36
III	239.99		
IV	297.14	1.24	2.38
V	377.14	1.27	
VI	445.711	1.112	6.86

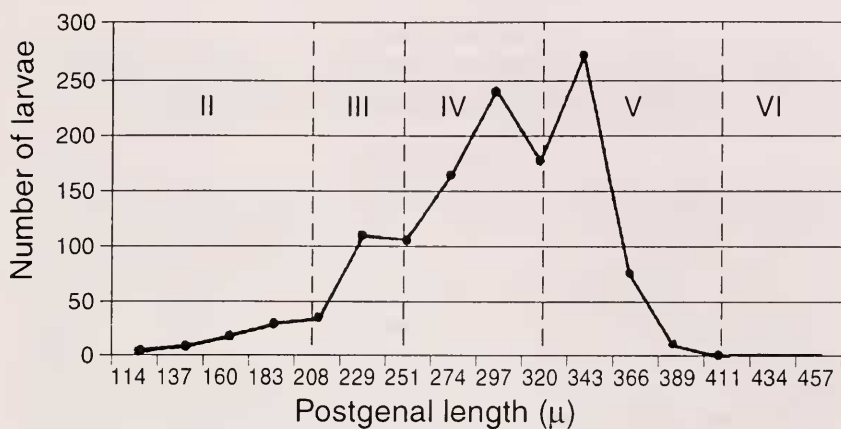


Fig. 2. Frequency distribution of the postgenal length of *S. (P.) haematopotum* (n = 1203).

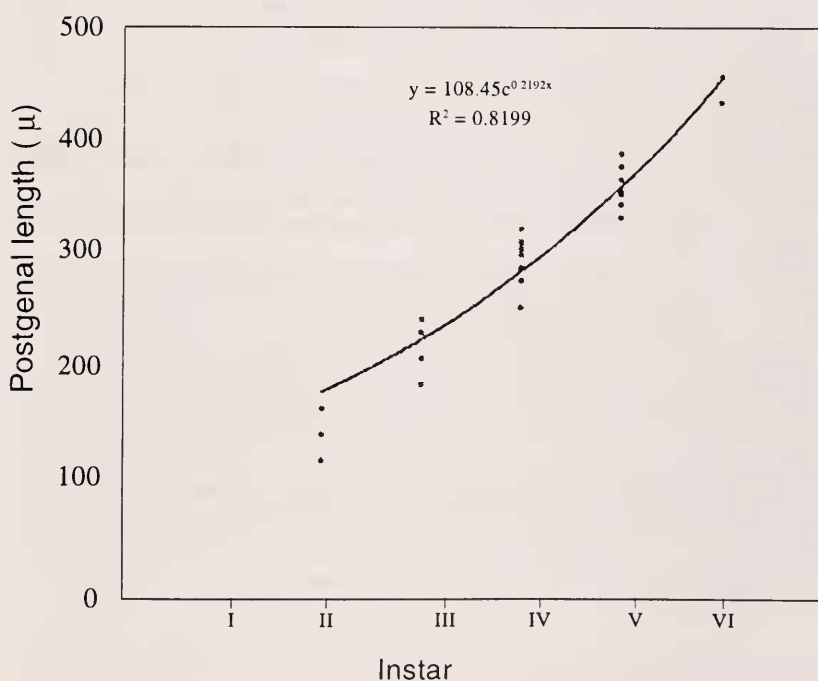


Fig. 3. *S. (P.) haematopotum*, representation of the postgenal length, in microns, for instars (means +/- standard deviation) and the adjusted regression lines.

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